Cell Comb™ Scratch Assay

Catalog No. 17-10191

(Patent Pending)
FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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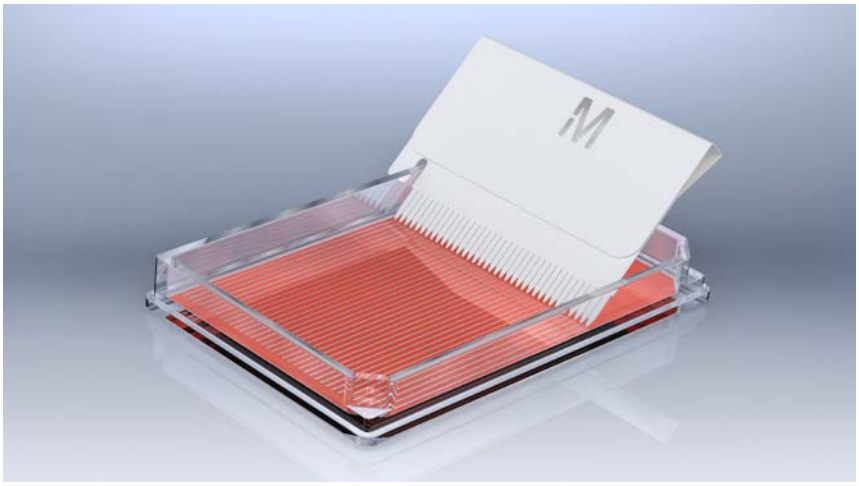
Introduction

Cell migration can be studied by a variety of methods, but the scratch assay remains a highly popular method due to the simplicity of the required materials, experimental setup, data collection and interpretation[1,2]. The scratch assay is typically initiated by scratching a confluent cell monolayer with a pipette tip to create a narrow wound-like gap. Shortly after wounding, the cells at the edge of the wound initiate a program to migrate into the gap, a process that continues until the gap has been completely repopulated with cells. Extent of wound closure is typically observed through light microscopy and protein expression patterns that occur during the wound healing process can be characterized by immunofluorescence.

Advances in understanding the repair mechanisms of wounded cell monolayers have been facilitated by the development of methods for performing the assay in multiwell plates. Such methods include delivering wounds to existing monolayers in the wells, or occlusion of the center of the well during monolayer formation to create a gap[3-5]. Each method then involves quantification of the extent of cell migration into the gap.

However, these methods are not optimal for biochemical analysis of the molecular events mediating wound repair. For example, the small scale of a basic pipette tip-derived wound provides insufficient and inadequate material for biochemical analysis. Using the same pipette tip to scratch a cell monolayer in a larger plate is tedious and irreproducible, and the proportion of migrating cells to quiescent cells is low. Several methods have been described to scale up the scratch assay by creating multiple wounds in cell monolayers but these methods require specialized tools[6,7].

EMD Millipore has developed the Cell Comb™ Scratch Assay to address the need for an easy-to-use tool for creating multiple scratch wounds, The Cell Comb™ has been optimized to apply a high density field of scratches to maximize the area of wound edges, while leaving sufficient numbers of undamaged cells to migrate into the gap. This form of high density wounding creates a high proportion of migrating cells to quiescent monolayer cells, which permits sensitive detection of the biochemical events occurring, specifically in the migrating cell population.



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Kit Components

1. Cell Combs (Part No. CS210543): Quantity of 6 individually packaged, disposable combs.

2. Rectangular Cell Culture Plates (Part No. CS210544): Quantity of 6 individually packaged, cell culture-treated 86 mm x 128 mm plates.

   The Cell Combs and Cell Culture Plates have been subjected to E-beam irradiation in order to minimize the possibility of contamination.

Materials Not Supplied

1. Sterile cell culture hood
2. Pipettes, liquid aspirators, etc. for handling of cells and liquid reagents
3. Sterile plastic ware (cell culture flasks, centrifuge tubes, pipettes, pipette tips, etc. for handling of cells and liquid reagents)
4. Sterile Dulbecco's phosphate-buffered saline (DPBS), without calcium or magnesium
5. Cell type of interest, with appropriate growth medium and cell detachment buffer (e.g., 0.25% trypsin)
6. Hemocytometer (e.g. Scepter™ Handheld Automated Cell Counter)
7. Trypan blue or equivalent viability stain
8. Low speed centrifuge for cell harvesting
9. CO_2 tissue culture incubator
10. Cell lysis buffer, compatible with biochemical assay of interest
11. Cell scraper

Storage & Usage

Store Cell Combs and Cell Culture Plates at room temperature. Use within **1 year** from date of receipt.

Before scratching a cell monolayer, practice running the comb across a clean plate.

Assay Protocol

1. Plate 10 mL cells of interest, suspended in growth media at a density that will yield a confluent monolayer within 1-2 days after plating, into the provided rectangular plates. Put the plates into an incubator with appropriate settings (usually 37°C, 5% CO_2).

   Note: Use caution to keep plates level while transporting in and out of the incubator, as rectangular plates are more prone to spillage of media than circular culture dishes.

2. When cells are confluent, remove media from the plate.
3. In a laminar flow hood, use scissors to open the pouch containing the Cell Comb. Remove Cell Comb™ from pouch, while handling only the top folded edge, and grasp the Cell Comb™ at the top folded edge, as shown in **Figure 1-A**.
4. Align the guide groove with the long edge of the plate, and draw the teeth of the comb across the monolayer, as shown in **Figure 1-B**. Apply sufficient force to apply even scratches on the monolayer, but **avoid excessive force that may bend the teeth**.

5. (Optional) To create scratches in 2 directions, align the guide groove with the short edge of the plate, and draw the teeth of the comb across the monolayer, perpendicularly to the first set of scratches, as shown in **Figure 1-C**
6. Discard comb after scratching one plate. Combs are designed for single use on one plate. **Figure 2** shows an example of the pattern of scratches made with the Cell Comb™.
7. Wash the scratched monolayer 1-2 times with media to remove detached cells, and leave 10 mL media in the plate.
8. Perform steps 2-8 for each additional plate of cells. It is recommended to include an unscratched plate of cells as a control.
9. Return the plates to incubator for desired time.

   Figure 3 depicts the time course of several key biochemical events, including Rac activation and phosphorylation of Erk and FAK.
10. After desired time period, remove the media and gently wash the cells with PBS. Collect or lyse cells in a manner appropriate for the intended biochemical analysis.

Example Data

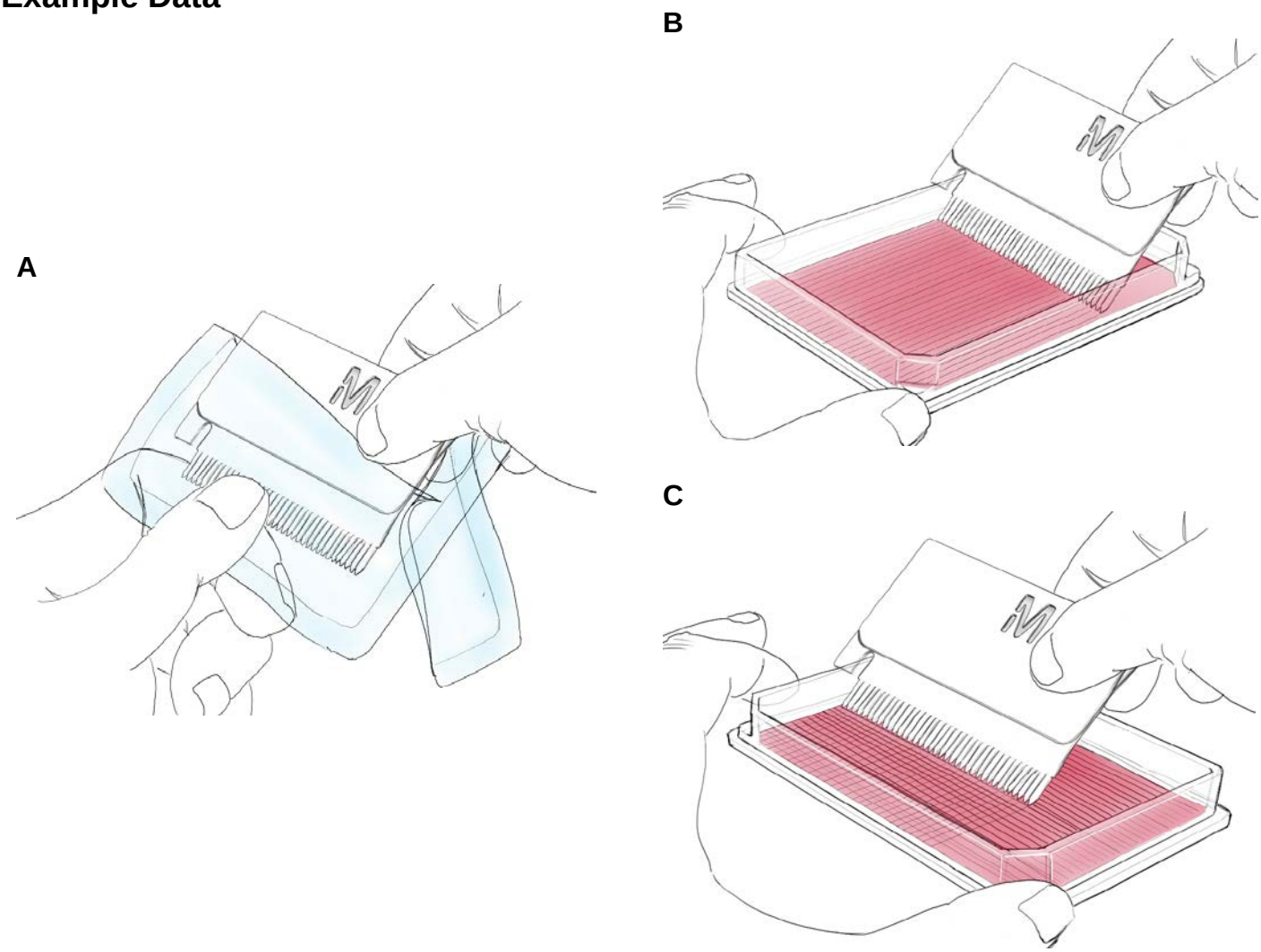

Figure 1. Usage Schematics
A) Removing the comb from the pouch.
B) Holding comb and drawing across a plate in the long direction (one-direction scratches).
C) Holding comb and drawing across a plate in the short direction (two-direction scratches).

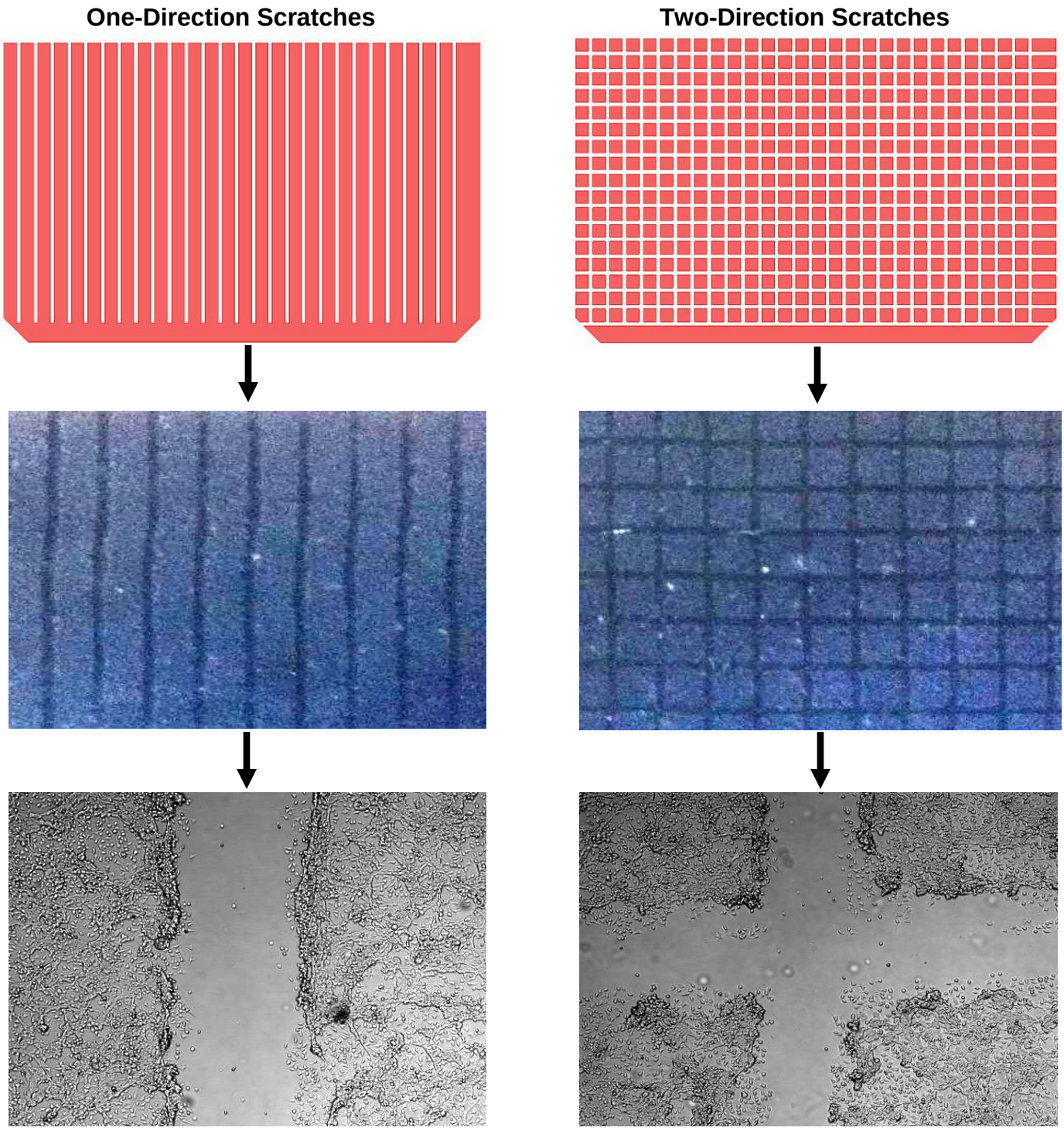

Figure 2. Schematic and photos of scratch pattern on a cell monolayer.
Top, drawing of scratch pattern in one-direction (*left*) and two-directions (*right*). *Middle,* 2X magnification view of cell monolayers with multiple wounds applied in one direction (*left*) or two directions (*right*) with the Cell Comb™. *Bottom*, phase contrast image (10X) of scratched cell monolayers immediately after wounding.

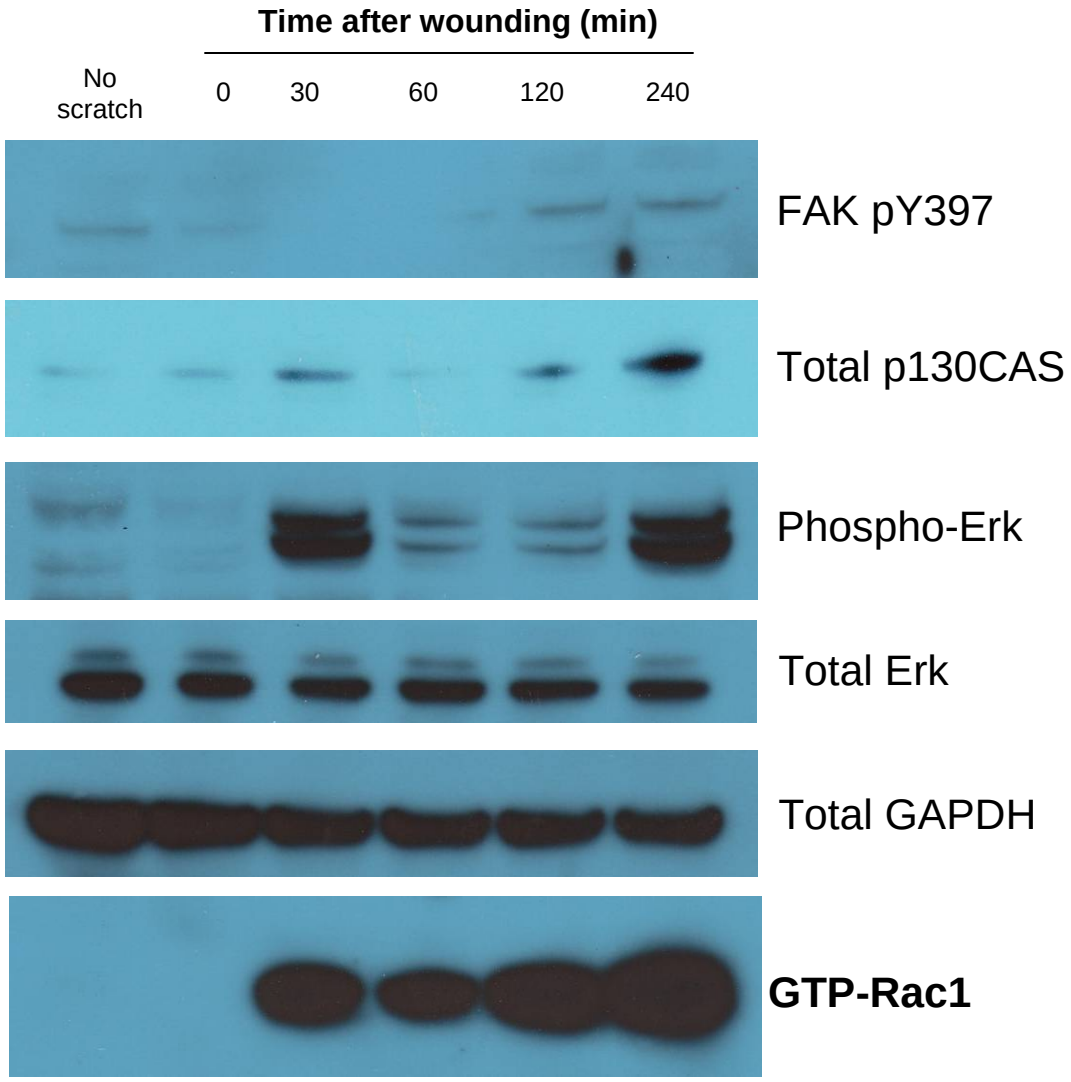

Figure 3. Biochemical analysis of cytoskeletal signaling after wounding with the Cell Comb™. A confluent monolayer of NIH3T3 cells in the Rectangular Cell Culture Plate was left intact or wounded with a Cell Comb™, and lysed after the indicated time. Lysates were analyzed by Western blot for phospho-FAK (Y397) (EMD Millipore Catalogue No. 05-1140), total p130Cas (EMD Millipore Catalogue No. 06-500), phospho-Erk (T202/Y204 and T185/Y187) (EMD Millipore Catalogue No. 05-797R), total Erk (EMD Millipore Catalogue No. 05-1152), and total GAPDH (EMD Millipore Catalogue No. MAB374).

Major increases in phosphorylated Erk are observed to occur in a bimodal fashion at 30 min and 4 hours after wounding; such a bimodal increase has been previously described[6]. At 2-4 hours post-wounding, increases in phosphorylated FAK and total pCas are observed.

In addition, lysates were analyzed for the GTP-bound form of Rac1 GTPase by pulldown assay with immobilized PAK1 (EMD Millipore Catalogue No. 17-441). In quiescent cells, activated Rac1 is undetectable, whereas within 30 minutes of wounding, a large increase in activated Rac1 is evident. In sum, major increases in cytoskeletal signaling events, such as Rac1 activation and Erk phosphorylation, are detected by biochemical analysis in cells wounded with the Cell Comb™ Scratch Assay.

References

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7. Lauder, H. *et al.* (1998) Quantification of the repair process involved in the repair of a cell monolayer using an in vitro model of mechanical injury. *Angiogeneisis.* 2: 67-80.

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